Polyunsaturated Fatty Acid Enrichment Increases Ultraviolet A–Induced Lipid Peroxidation in NCTC 2544 Human Keratinocytes

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The influence of cell enrichment with fatty acids with increasing degree of unsaturation on the ultraviolet A–induced formation of lipid-peroxidation products (thiobarbituric acid reactive substances [TBARS]) has been investigated in NCTC 2544 human keratinocytes. A 48-h preculture of cells in controlled medium supplemented with unsaturated fatty acids resulted in a marked increase in TBARS appearance under ultraviolet A exposure. This effect was dependent upon the degree of unsaturation of the fatty acids, with the following order of efficiency: arachidonic > linolenic > linoleic > oleic acid. For arachidonic acid (AA), the potentiating effect on ultraviolet A–induced lipid peroxidation was dependent upon the fatty acid concentration, with about a 2.5-fold increase in TBARS formation in cells pre-cultured with 5×10^{-5} M AA, then exposed to a UVA dose of 13 J/cm^2. The increase in TBARS formation by AA was almost totally prevented by supplementation of cells with 5×10^{-5} M vitamin E, whereas buthionine sulfoximine, a chemical which depletes cell glutathione, potentiated lipid peroxidation. These results suggest that the nature of the fatty acids of cellular lipids could influence the response of keratinocytes to ultraviolet A, and especially the ultraviolet A–induced lipid peroxidation. *J Invest Dermatol 104:964–969, 1995*

The role of solar ultraviolet (UV) radiation in photoaging and photocarcinogenesis is now well recognized [1,2]. The effects of ultraviolet B (UVB, e.g., wavelength in the range 290–320 nm) have been first extensively studied, because crucial cellular targets such as nucleic acids are directly damaged by these radiations [3,4]. However, recent studies on cultured cells have shown that ultraviolet A (UVA, e.g., wavelength in the range 320–400 nm) can also induce cellular damage, most probably via photosensitized reactions involving endogenous photosensitizers such as flavines or NADH/NADPH [5,6]. The irradiation of cultured cells with UVA has been shown to activate phospholipase A_2 [7] and to increase protein kinase C activity [8]. It has also been demonstrated that UVA radiations induce cellular lipid peroxidation in cultured fibroblasts [9] and keratinocytes [10]. The fatty acid composition of skin cell membranes could be expected to influence the susceptibility of lipids to peroxidation. We therefore investigated the consequences of the supplementation of cultured keratinocytes with arachidonic acid, a highly peroxidable fatty acid, on the UVA-induced formation of lipid peroxidation products. Our results demonstrate that enrichment of cultured human keratinocytes with unsaturated fatty acids, and especially arachidonic acid, results in a marked increase in lipid peroxidation upon UVA exposure. It is concluded that the fatty acid composition of lipids could influence the susceptibility of skin cells to damage induced by UV radiations.

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Abbreviations: BSO, buthionine sulfoximine; FAME, fatty acid methyl esters; TBARS, thiobarbituric acid reactive substances.

MATERIALS AND METHODS

Materials The NCTC 2544 human keratinocyte cell line [11] was purchased from Flow (Paris, France). Dulbecco's modified Eagle's Medium (DMEM), Hanks' salt solution, and fetal bovine serum were provided by Gibco (Grand Island, NY). Arachidonic, linoleic, linolenic, stearic, and palmitic acids, human free fatty acid serum albumin, and all other reagents were purchased from Sigma (St. Louis, MO), and were of the purest available grade.

Cell Culture Cells were seeded at a density of 10^4/cm^2, and cultured in 35-mm Nunc Petri dishes in DMEM medium supplemented with 10% fetal bovine serum. Cultures were maintained at 37°C in a 5% CO_2 humidified atmosphere. All experiments were carried out 3 d after cell seeding. For the gas chromatographic determination of the fatty-acid composition of cells, NCTC 2544 keratinocytes were seeded at the usual density (10^4/cm^2), but were cultivated in 60-mm Petri dishes (Nunc). In some experiments (see below), the influence of cell density on the UVA-induced formation of lipid peroxidation end products has also been investigated. To this purpose, cells were seeded at densities varying from 10^4 to 5×10^5/cm^2.

Fatty Acid Supplementation of Cultured Cells For the modification of the fatty acid composition of cellular lipids, the keratinocytes were cultured for 48 h in DMEM supplemented with oleic, linoleic, linolenic, or arachidonic acid (AA) as previously described [12]. The fatty acid was

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Table I. Fatty Acid Composition of NCTC 2544 Keratinocytes Cultured in Media Enriched with Various Fatty Acids[a]

	14:0	16:0	16:1, n-7	18:0	18:1, n-7	18:1, n-9	18:2, n-6	18:3, n-6	20:1, n-6	20:1, n-9	20:3, n-6	20:3, n-9	20:4, n-6	20:5, n-3	22:0	22:1, n-9	22:6, n-3	24:0
Controls	0.39	17.31	12.6	6.9	13.5	36.1	4.2	ND	0.13	0.22	0.17	0.88	5.2	1.0	0.22	0.22	0.17	0.33
18:1, n-9	0.36	14.9	10	7.1	12.7	42	4.0	ND	0.15	0.33	0.13	0.82	5.7	0.88	0.24	0.13	0.13	0.35
18:2, n-6	0.44	15.1	9.4	8.8	9.2	31	16.6	1.9	0.14	0.21	0.14	0.37	5.2	0.42	0.33	0.11	0.13	0.47
18:3, n-6	0.41	14.8	9.3	10.9	6.2	28.8	5.3	3.4	0.11	0.21	0.48	0.27	18.1	0.32	0.38	0.11	0.11	0.55
20:4, n-6	0.58	13.6	8.1	11.5	7.0	31	4.8	ND	0.11	0.15	0.12	0.30	21.4	0.32	0.34	0.12	0.13	0.47

[a] Results are expressed as means (four experimental values, two experiments in duplicate) of the percentage of fatty acids present in the total extract (see *Materials and Methods*). For the sake of clarity, SD is not given, but did not exceed ±15%. ND, not done.

resuspended in 0.2% free fatty acid human serum albumin. In most experiments, the final concentration of the fatty acid in culture medium was 5×10^{-5} M. In experiments dealing with the effect of the fatty acid concentration on the UVA-induced formation of TBARS, the fatty acid concentration varied from 10^{-5} to 10^{-4} M.

Assessment of the Fatty Acid Composition of Keratinocytes The modification of the fatty acid content of cellular lipids was assessed by gas liquid chromatography coupled to mass spectrometry. Following the 48-h culturing in medium supplemented with fatty acids (see above) cells were washed three times with phosphate-buffered saline supplemented with 0.2% bovine serum albumin, harvested with a rubber policeman and centrifuged for 5 min at $400 \times g$. The cell pellet was then extracted by the method of Folch *et al* [13] and lipids evaporated to dryness under nitrogen. The fatty acid hydrolysis and derivatization was performed as described by Ponec *et al* [14]. The fatty acid composition of cellular lipids was then assessed by gas chromatography coupled to mass spectrometry. One tenth of the fatty acid methyl esters (FAME) in hexan was injected into the gas chromatograph (DI700, Delsi Instruments) coupled to a Delsi-Nermag R10-10C mass spectrometer via a Ross falling needle injector (capillary column Supelcowax 10, 30 m, 0.32 μm internal diameter, 0.25 mm film thickness). Helium was used as a carrier gas. A good resolution of the FAME was obtained when the oven temperature of the gas chromatograph was programmed between 185°C and 201°C at 1°C/min and between 201°C and 230°C at 10°C/min. The detection of the eluted FAME was performed in the Chemical Ionization Mode of the mass spectrometer with ammonia (0.1 Torr) as the reagent gas, which provides a sensitive and quantitative evaluation of the different FAME based on the M + 18 adduct. The results were obtained after time integration in the chromatogram of the ion mass selected and final processing of the peak areas by Excel (Microsoft).

Experimental Conditions for Cell Irradiation Before UVA irradiation, cells were washed once with Hanks' salt solution supplemented with 0.2% human free fatty acid serum albumin, then twofold with Hanks' salt solution devoid of albumin. The UVA irradiation was performed at 365 nm in 1 ml Hanks' salt solution devoid of any additive, with a Vilber Lourmat (Torcy, France) table equipped with TF-20L tubes and appropriate filters [15,16]. A glass window (4 mm thickness) was placed above the lamp to absorb the remaining UVB light of the commercial equipment (transmittance < 0.01% at 310 nm). Samples (plastic Petri dishes) were placed on the glass window and irradiated from the bottom. The central area of the table allowed the homogenous irradiation of 15 Petri dishes. An average fluence rate of 3.5 ± 0.2 mW/cm^2 was measured with the Vilber Lourmat UVR 365 photometer. After irradiation, cells were left in the dark for 1 h at 37°C. Sham-irradiated cells were left under similar conditions but without UVA exposure.

TBARS Measurement Lipid peroxidation products (TBARS) were measured at the end of the 1-h dark period following cell irradiation. Measurements were performed on an aliquot of the irradiated medium, because it has been previously shown that most (more than 80%) of the TBARS produced after UVA exposure are secreted by cells [9]. In some experiments, the effect of the antioxidant vitamin E and of the pro-oxidant compound buthionine sulfoximine (BSO) on the UVA-induced lipid peroxidation were studied in control cells or in cells enriched with arachidonic acid. To this purpose, cells were cultured 24 h before irradiation either with 5×10^{-5} M vitamin E + 10^{-3} M vitamin C, or with 10^{-5} M BSO. The thiobarbituric acid reactive substances were determined by the fluorometric method described by Yagi [17]. The results were calculated as nmol MDA equivalent produced/mg cell protein.

Protein determination was done by the method of Lowry modified according to Peterson [18].

Assay of Cell Viability Cell viability was assessed 24 h after irradiation by the Neutral Red assay. To this purpose, cells were washed three times with PBS, and incubated for 4 h in the presence of 1 ml of the dye diluted in PBS [19]. At the end of the incubation time, cells were washed three times with PBS, and the optical density was read at 535 nm. Results are given as percentages of controls (unirradiated cells, time zero).

All experiments were performed at least in triplicate. Statistical analysis was performed with the Student t test.

RESULTS

Assessment of the Fatty Acid Composition of Lipids in Cells Precultured in Medium Enriched with Unsaturated Fatty Acids The results in **Table I** show the influence of NCTC 2544 keratinocyte supplementation with oleic (18:1, n-9), linoleic (18:2, n-9), gamma-linolenic (18:2, n-6), or arachidonic (20:4, n-6) acids on the fatty acid composition of cellular lipids. It must be first noted that in our experimental conditions (no serum, and cell cultured in the presence of fatty acid–albumin complexes) a particular fatty acid profile was observed in control cells, characterized by relatively high levels of palmitoleic (16:1, n-7) and (18:1, n-7) acids. Moreover, the presence in significant amounts of eicosatrienoic acid (20:3, n-9) was observed. This reveals a relative carency in polyunsaturated fatty acids. These conditions—cells cultured for 48 h in medium supplemented with free fatty acid serum albumin [12,20,21]—were chosen to control more precisely the cellular fatty acid composition and to obtain more marked enrichment of cellular lipids as compared to cultures in which the fatty acids are introduced in medium supplemented with serum [22]. Thus the fatty acid composition of cells cannot be compared to that previously reported in human keratinocytes cultured in the presence of whole fetal bovine serum [23]. It must be emphasized that the purpose of this analysis was mainly to check to which amount the cellular lipids were enriched in the fatty acid species introduced in the culture medium. To this point of view, results in **Table I** effectively demonstrate that cell culture in the presence of each of the chosen fatty acids resulted in a marked increase in its respective percentage (from 36.1% to 42%, 3% to 15%, traces to 3.4%, and 5.2% to 21.4% for oleic, linoleic, linolenic, and arachidonic acids, respectively). It is of note that after irradiation (13 J/cm^2) a significant decrease in the AA percentage was observed both in control and AA-enriched cells (about −15% in controls and −25% in cells cultured in the presence of AA, data not shown). This demonstrates AA consumption upon UVA exposure, most probably by oxidation, which could explain the major increase in TBARS formation in AA-enriched cells (see below).

Influence of the Degree of Unsaturation of the Fatty Acids on the UVA-Induced Lipid Peroxidation Previous studies on fibroblasts [9,15] or keratinocytes [10] have shown that UVA exposure is accompanied by a dose-dependent formation of lipid peroxidation end products (TBARS), mainly malondialdehyde originated from unsaturated fatty acid degradation [9]. The results in **Fig 1** indicate that the extent of TBARS formation increased with the degree of unsaturation of the fatty acid added to the medium. Under our experimental conditions (fatty acid concentration of 5×10^{-5} M and UVA dose of 13 J/cm^2), the TBARS formation was about 2.5-, 2-, and 1.4-fold increased by arachidonic, gamma-linolenic, and linoleic acid, respectively. It must be noted that oleic acid (18:1), which is the naturally most represented fatty acid in cells [22], did not significantly affect the UVA-induced

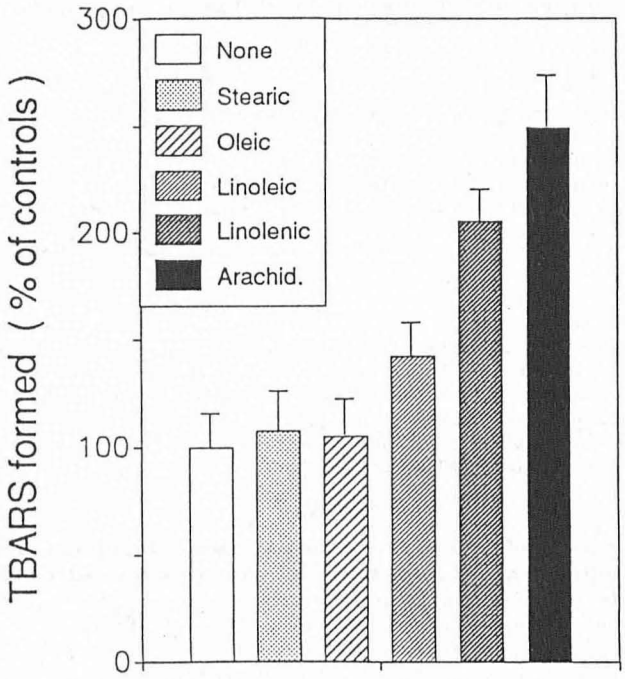

Figure 1. Influence of the degree of unsaturation of the fatty acids on the UVA-induced TBARS formation in NCTC 2544 keratinocytes. Cells were precultured for 48 h in media supplemented with 5×10^{-5} M of either stearic, oleic, linoleic, linolenic, or arachidonic acid, then exposed to a UVA dose of 13 J/cm^2. Each point represents the mean of six experimental values ±SD. 100% = 0.85 ± 0.12 nmol MDA equivalent/mg cell protein.

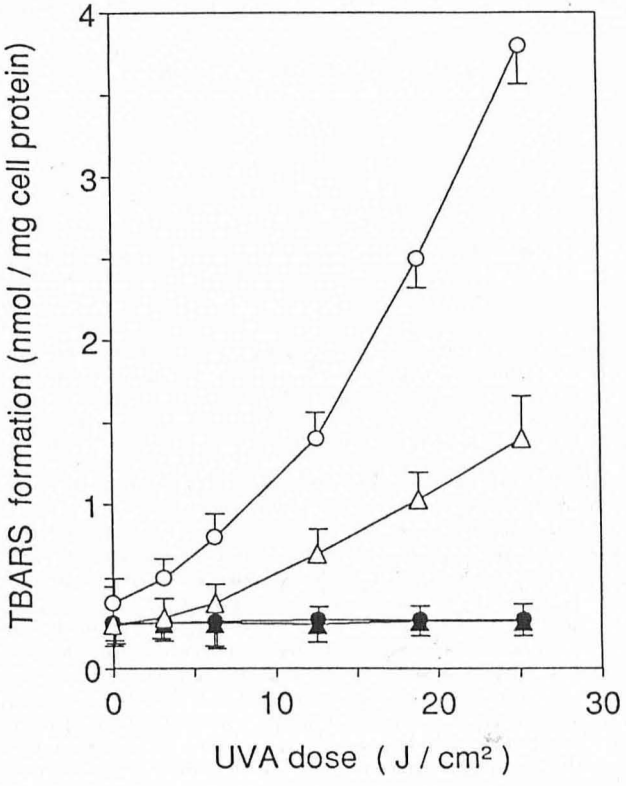

Figure 2. Influence of arachidonic acid (AA) enrichment on the UVA-induced TBARS formation in NCTC 2544 keratinocytes as a function of the irradiation dose. The cells were irradiated in Hanks' salt solution as specified in *Materials and Methods*. After irradiation, cells were left for 1 h at 37°C in the CO_2 incubator. The UVA-induced TBARS formation was then measured on the incubation medium by the fluorometric method of Yagi. *Closed symbols*, sham-irradiated cells; *open symbols*, irradiated cells. *Triangles*, control (unsupplemented) cells; *circles*, cells precultured for 48 h in the presence of 5×10^{-5} M AA. Each point represents the mean of six experimental values ±SD.

lipid peroxidation, as compared to control cells. It is of interest to note that enrichment of cells with stearic acid, which is devoid of any double bond, did not significantly alter the TBARS production upon UVA exposure (data not shown). Another interesting observation is that gamma-linolenic acid (18:3, n-6) was almost as efficient as arachidonic acid in potentiating the UVA-induced TBARS formation. This can be explained by the fact that 18:3, n-6 is actively converted in arachidonic acid (see **Table I**), which demonstrates the existence of a delta 5 desaturase activity in NCTC 2544 keratinocytes under our experimental conditions.

Time and Dose Dependency of the Effect of AA Enrichment on UVA-Induced Lipid Peroxidation Further experiments were carried out using the most effective of the fatty acids, arachidonic acid (AA), to specify the time and dose dependency of the phenomenon. **Figure 2** shows that the enrichment of cells by a 48-h preculture in the presence of 5×10^{-5} M AA led to a marked increase in UVA-induced lipid peroxidation, with about threefold more TBARS formed as compared to controls for a UVA dose of 19 J/cm^2. While in control cells, very weak production of TBARS was noted below 6 J/cm^2, an about twofold increase of the basal TBARS level was already observed in AA-supplemented cells as compared to sham-irradiated cultures.

The influence of the fatty acid concentration on the TBARS formation upon UVA irradiation has also been investigated. **Figure 3*a*** shows that the TBARS formation increased up to a concentration of 7.5×10^{-5} M, at which the enhancing effect of arachidonic acid on UVA-induced lipid peroxidation plateaued. Similar results were obtained when the TBARS generated upon UVA exposure were expressed as a function of the percentage of arachidonic acid recovered in cellular lipids, as shown in **Figure 3*b***.

Effect of Cell Density on TBARS Formation Observations in our laboratory indicated that cultured fibroblasts or keratinocytes appeared to be morphologically more alterated by UVA radiation when the cell density was low as compared to subconfluent cells. We therefore investigated the influence of the cell-seeding density on TBARS formation in control or AA-enriched NCTC 2544 cultures exposed to UVA. The results displayed in **Fig 4** demonstrate that the UVA-induced TBARS formation was inversely correlated to the cell-seeding density. Again, AA-enrichment (5×10^{-5} M) of the culture medium resulted in a large increase in TBARS formation upon UVA exposure.

Effect of Anti- and Pro-Oxidant Compounds The effect of antioxidants (vitamin E + vitamin C) and of the pro-oxidant compound BSO, which is known to reduce the cellular glutathione [24], was also investigated on UVA-induced lipid peroxidation. **Figure 5** shows that either in control cultures or in cultures supplemented with AA, antioxidants strongly reduced (70–80%) the TBARS formation, whereas BSO potentiated it by 1.3- to 1.5-fold. It is noteworthy that neither vitamin E nor BSO had a major effect on the initial arachidonic percentage of NCTC 2544 lipids **(Table II)**. However, a slight increase in AA content was observed in irradiated cells precultured with vitamin E, whereas the opposite phenomenon was observed in BSO-treated cells. This can be explained if we admit that AA is a major substrate for lipid peroxidation processes, which also minimally occurred in normal conditions.

Relationship Between UVA Exposure and Cell Viability in AA-Enriched Cells The effect of UVA exposure on the cell viability, as assessed by the Neutral Red uptake test, is shown in **Fig 6**. It can first be observed that arachidonic acid supplementation did not significantly alter cell viability in unirradiated cells. For a UVA

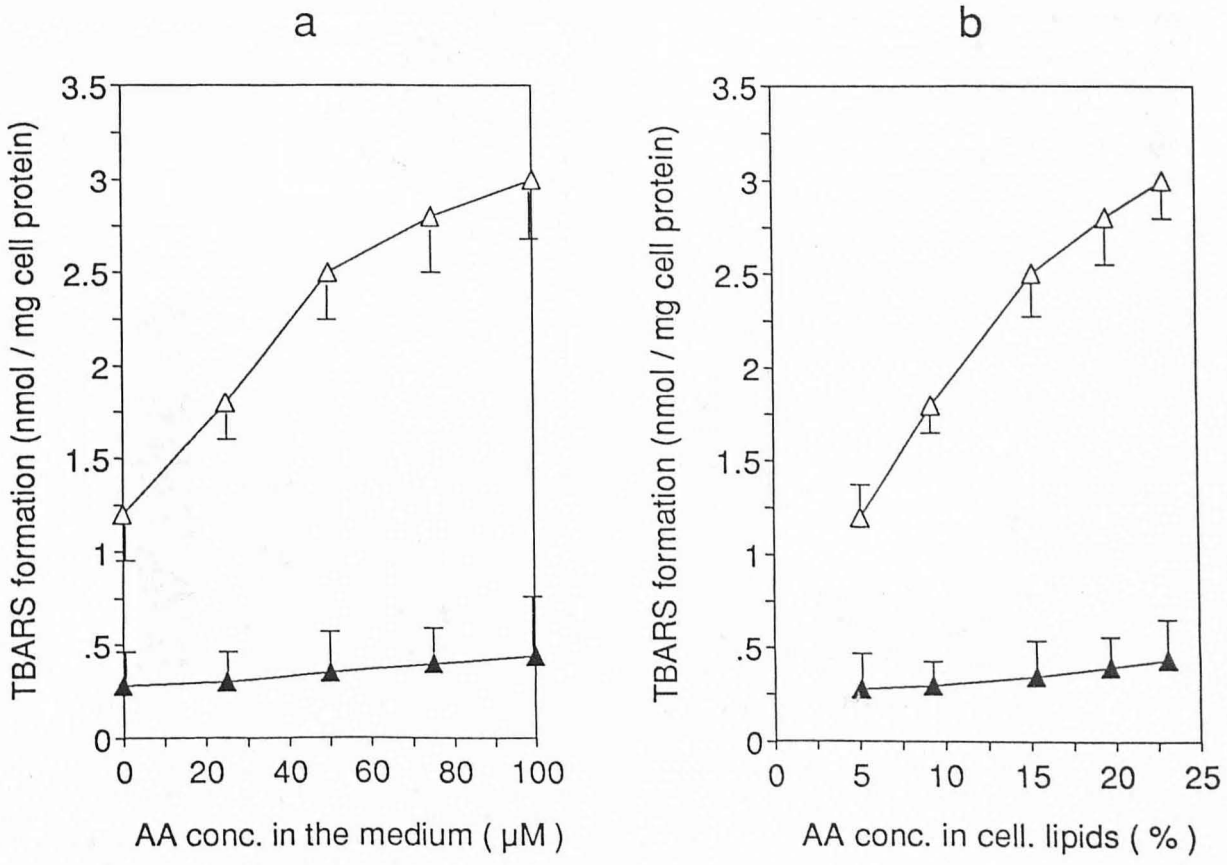

Figure 3. Influence of arachidonic enrichment of NCTC2544 keratinocytes on the UVA-induced TBARS formation as a function of the fatty acid concentration in the culture medium (*a*) or in cellular lipids (*b*; in this case, the AA concentration was expressed as percentage of the fatty acids present in cellular lipids, see Table I). The experimental conditions were the same as described in the **Fig 1** legend, except that the arachidonic concentration in the culture medium varied from 0 to 10^{-4} M, whereas the UVA irradiation dose was fixed at 13 J/cm^2. Each point represents the mean of four experimental values ±SD. *Closed symbols*, sham-irradiated cells; *open symbols*, irradiated cells.

dose of 13 J/cm^2, the cell viability measured 24 h after irradiation was only 15–20% reduced in control (unsupplemented) cells. In arachidonic acid–supplemented cells, a dose-dependent decrease in cell viability was observed, with about 45% and 70% reduction in Neutral Red uptake for 5×10^{-5} and 7.5×10^{-5} M AA, respectively. It must be noted that when the Neutral Red test was performed 1 h after irradiation, the dye uptake was much less impaired, with only 25–30% reduction in cells supplemented with 7.5×10^{-5} M AA (data not shown). Thus cellular damage triggered by UVA irradiation in AA-supplemented cells is amplified with incubation time in the dark, resulting in a marked photocytotoxic effect after 24 h. This suggests a possible relationship with the auto-propagated lipid oxidative processes.

DISCUSSION

In this work, we tested the hypothesis that a change in the fatty acid composition of cellular lipids might influence the appearance of lipid-peroxidation products upon UVA exposure. Our results clearly demonstrate that the enrichment of NCTC 2544 keratinocytes with unsaturated fatty acids resulted in an enhancement of the formation of lipid-peroxidation products by UVA. This effect was proportional to the degree of unsaturation of the fatty acid and to its concentration. The relationship between lipid peroxidation and the fatty acid unsaturation clearly appears if we compare the results obtained with linoleic (two double bonds) and arachidonic (four double bonds) acids. For a similar (about fourfold) increase in the percentage of each fatty acid after supplementation (see **Table I**), 1.3- and 2.5–3-fold increases in TBARS formation were observed, respectively **(Fig 1)**. The TBARS formation was prevented by the lipid-peroxidation chain breaker vitamin E, either in control cultures or in cultures enriched in AA. It can therefore be concluded that the UVA-induced lipid peroxidation can be influenced by the fatty acid composition of cellular lipids. Albeit this result might be theoretically expected, this is to our knowledge the first demonstration of the possibility to influence the UVA-induced peroxidation of cellular lipids by manipulating their fatty acid composition.

It is also of note that the TBARS formation upon UVA exposure was inversely correlated to the cell-seeding density (see **Fig 4**). This suggests that cells seeded at a low density, which were in exponential growth at the time of experiments, had lower antioxidant defenses than subconfluent cells. The UVA exposure also more markedly affected the cell viability in sparse cultures as compared to dense cultures (data not shown). Such a phenomenon has been previously described in another experimental model: human fibroblasts exposed to oxidized low-density lipoproteins [25]. The authors concluded that cells are more sensitive to the oxidative stress during the S phase of the cell cycle [25]. Recently, Gaboriau *et al* also found that the cellular damage induced by UVA in cultured fibroblasts, as assessed by the release of lactate dehydrogenase activity in the irradiation medium, was more pronounced in cells in exponential growth [26]. Taken together, these observations suggest that the keratinocytes of the basal layer of the skin, which are actively dividing cells, could be more sensitive to UVA-induced lipid peroxidation and cellular damage than more differentiated keratinocytes.

The UVA radiations have been shown to induce lipid peroxidation in fibroblasts [9,15,16] or keratinocytes [10]. The lipid peroxidation products (hydroperoxides, aldehydes) alter the activity of numerous enzymes controlling the cellular intermediary metabolism and of membrane-bound enzymes and ion transporters

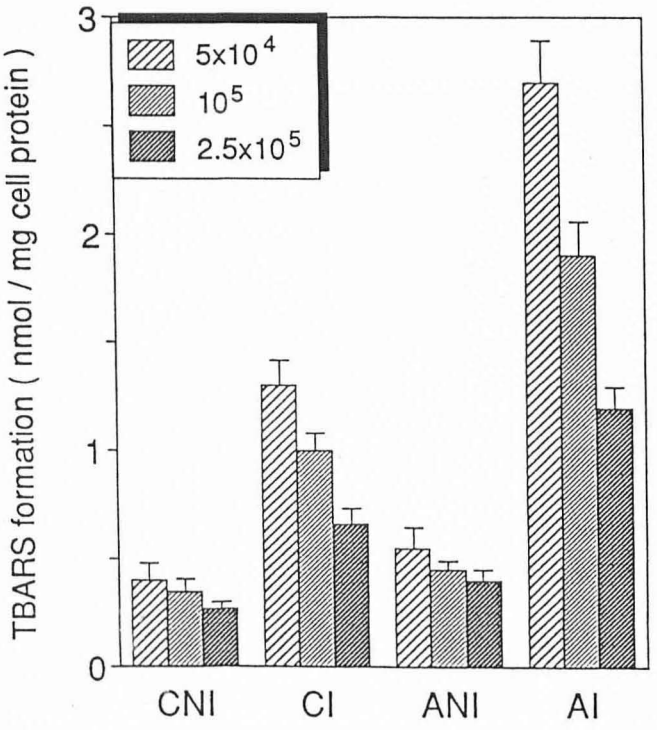

Figure 4. Influence of cell-seeding density on TBARS formation in control and arachidonic acid (5×10^{-5} M)-enriched NCTC 2544 human keratinocytes. The experimental protocol was the same as described in the **Fig 3** legend. CNI, sham-irradiated cells cultured; CI, cells irradiated with a UVA dose of 13 J/cm^2; ANI, sham-irradiated cells enriched with AA; AI, cells enriched with AA and then irradiated with a UVA dose of 13 J/cm^2. Each point represents the mean of six experimental values ±SD.

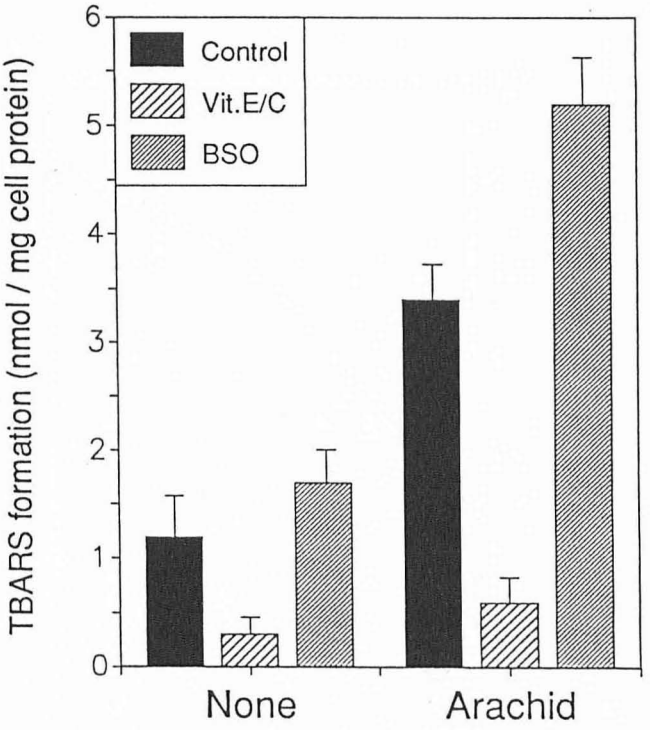

Figure 5. Effect of vitamin E and BSO on the UVA-induced TBARS formation in control or in arachidonic acid–enriched NCTC 2544 keratinocytes. The experimental protocol was the same as described in the **Fig 3** legend, except that cells were pre-cultured with either 5×10^{-5} M vitamin E or with 10^{-5} M BSO 24 h before irradiation. The irradiation dose was 13 J/cm^2. Arachidonic acid enrichment was achieved by a 48-h preculture in medium supplemented with the fatty acid at a final concentration of 5×10^{-5} M. None, cells cultured in standard conditions; AA, cells enriched with arachidonic acid. Each point represents the mean of four experimental values ±SD.

[27,28]. It is thus of importance to specify the factors that can influence the UVA-induced appearance of lipid peroxidation products in skin cells. Up to now, information on this topic was lacking. It is of interest to note that the fatty acid composition of tissue lipids can be modulated by changing the fatty acid balance in the diet, as it has been demonstrated by numerous studies performed on various experimental models [29–31]. The fatty acid composition of cellular lipids can also be modified *in vitro*, by using controlled media devoid of serum, a widely employed method [12,20,21]. This strategy has been used in the present experiments. The composition of cellular lipids may also be changed by incubation of cultured cells with phospholipid emulsions of defined composition [32,33]. It also may be supposed that topical application of phospholipid emulsions could influence the lipid composition of skin cells. Thus, the modulation of the response of skin cells to UV radiations by changing the nature of cellular lipids (and especially their fatty acid composition) appears to be an interesting way of research.

In the present studies, the *in vitro* enrichment of keratinocytes with unsaturated fatty acids resulted in an increased lipid peroxidation. This is *a priori* a negative effect, considering the potential toxicity of lipoperoxides. It is of interest to note that, under our experimental conditions (a relatively mild irradiation dose of 13 J/cm^2, which corresponds to about one third to one half of the minimal erythemal dose in subjects with phototype II) [34], there is only weak alteration of cellular viability at the minimal arachidonic acid concentration (2.5×10^{-5} M), as assessed by the Neutral Red test **(Fig 6)**. In fact, when submitted to mild oxidative stresses, up to a threshold dose that probably depends upon their antioxidant defenses, cells are in most cases able to survive [35,36]. However, even non-lethal doses of UVA have been shown to result in significant alterations in the activity of enzymes of the intermediary metabolism [37], in a decrease in growth-factor binding [8], and endocytotic processes [16]. In contrast, at higher (5×10^{-5} and 7.5×10^{-5} M) AA concentrations, a marked decrease in cell viability was observed. Thus an increase in the degree of unsaturation of cellular lipids should in principle enhance the susceptibility of skin to solar radiations and accelerated photodegenerative processes. However, it is difficult to predict from the present experiments the actual consequences of a change in the skin cell fatty acid composition on their *in vivo* response to UV radiations, because skin is a complex tissue, in which several types of cells with very different metabolisms are co-existing and even cooperating. Moreover, skin

Table II. Effect of Vitamin E (5×10^{-5} M) and Vitamin C (10^{-3} M) or BSO (10^{-5} M) on the Percentage of Arachidonic Acid (AA) in Total Lipids of NCTC 2544 Human Keratinocytes[a]

	AA Percentage
Controls	
No addition	4.7 ± 0.6
+ vitamin E/vitamin C	6.2 ± 0.8
+ BSO	5.1 ± 0.6
AA-enriched	
No addition	19.7 ± 2.6
+ vitamin E/vitamin C	21.5 ± 1.8
+ BSO	17.9 ± 2.1

[a] Antioxidants or BSO were added 24 h before lipid analysis (see *Materials and Methods*). Controls, cells cultured in the absence of AA; AA-enriched, cells cultured for 48 h in the presence of 5×10^{-5} M AA. Means of four experimental values (two experiments in duplicate) ±SD.

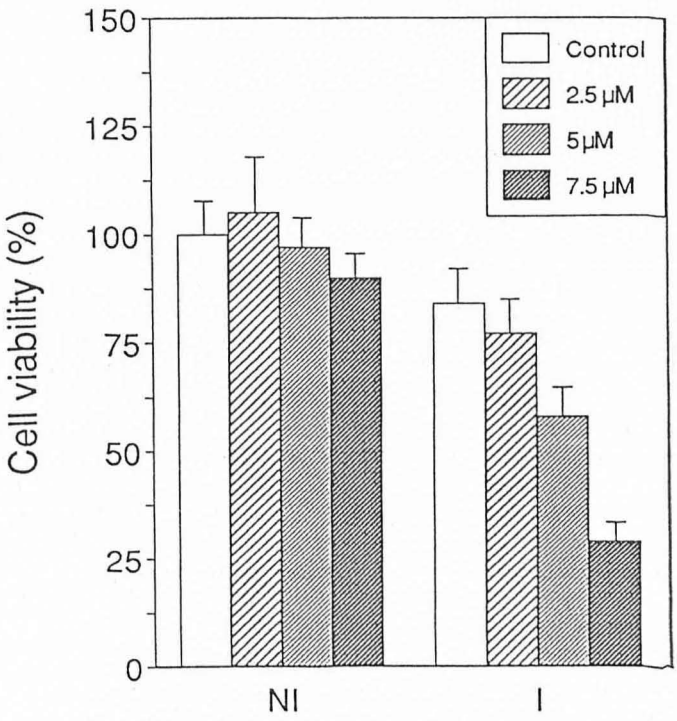

Figure 6. Effect of arachidonic acid enrichment on the viability of NCTC 2544 keratinocytes as a function of the fatty acid concentration. The experimental protocol was the same as described in the **Fig 2** legend. Twenty-four hours after irradiation at a fixed dose of 13 J/cm^2, the cell viability was assessed by the Neutral Red colorimetric assay (see *Materials and Methods*). NI, sham-irradiated cells; I, cells irradiated with a UVA dose of 13 J/cm^2. Each point represents the mean of six experimental values ±SD.

cells have different antioxidant defense capacities [38], which suggests that their response to fatty acid modification could be different, in terms of sensitivity to UV radiations. For example, preliminary studies in our laboratory have shown that cultured human fibroblasts are more sensitive to UVA-induced lipid peroxidation and arachidonic acid enrichment than human keratinocytes (data not shown). Further studies on animal models are therefore necessary to specify the eventual relationship between the changes in skin cell lipid composition and the response of skin to UV radiations.

Our study points at the potential importance of the fatty acid composition of cellular lipids on the UVA-induced lipid peroxidation. This has to be kept in mind in the choice of the lipid vectors used for cosmetic preparations, because it is conceivable that they can influence the sensitivity of skin cells to solar radiations, and thus the progress of skin photoaging.

REFERENCES

1. Diffey BD: Analysis of the risk of skin cancer from sunlight and solaria in subjects living in Northern Europe. *Photodermatol* 4:118–126, 1987
2. Urbach F: Potential effects of altered solar ultraviolet radiation on human skin cancer. *Photochem Photobiol* 50:507–513, 1989
3. Freeman SE, Gange RW, Sutherland JC, Sutherland BM: Pyrimidin dimer formation in human skin. *Photochem Photobiol* 46:207–212, 1987
4. Keyse SM, Tyrrell RM: Rapidly occurring DNA excision repair events determine the biological expression of UV-induced damage in human cells. *Carcinogenesis* 8:1251–1256, 1987
5. Moan J, Peak MJ: Effects of UV radiations on cells. *J Photochem Photobiol B: Biol* 4:21–34, 1989
6. Tyrrell RM, Keyses SM: New trends in photobiology of the interaction of UVA radiation with cultured cells. *J Photochem Photobiol B: Biol* 4:349–361, 1990
7. Hanson D, DeLeo VA: Long wave ultraviolet irradiation stimulates arachidonic acid release and cyclo-oxygenase activity in mammalian cells in culture. *Photochem Photobiol* 49:423–430, 1989
8. Matsui MS, DeLeo VA: Induction of protein kinase C activity by ultraviolet radiation. *Carcinogenesis* 2:229–234, 1990
9. Morlière P, Moysan A, Santus R, Hüppe G, Mazière JC, Dubertret L: UVA-induced lipid peroxidation in cultured human fibroblasts. *Biochim Biophys Acta* 1084:261–268, 1991
10. Punnonen K, Jansén CT, Puntala A, Ahotupa M: Effect of in vitro UVA radiation and PUVA treatment on membrane fatty acids and activities of antioxidant enzymes in human keratinocytes. *J Invest Dermatol* 2:255–259, 1991
11. Perry VP, Sanford KK, Evans VJ, Hyatt GW, Earle WR: Establishment of epithelial cells from human skin. *J Natl Cancer Inst* 18:707–717, 1957
12. Mazière C, Mazière JC, Mora L, Polonovski J: Incorporation of exogenous fatty acids into phospholipids by cultured hamster fibroblasts. *Biochim Biophys Acta* 712:712–715, 1982
13. Folch J, Lees M, Sloane Stanley GH: A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 226:497–509, 1957
14. Ponec M, Weerheim A, Kempenaar J, Mommaas AM, Nugteren DH: Lipid composition of cultured human keratinocytes in relation to their differentiation. *J Lipid Res* 29:949–961, 1988
15. Santus R, Perdrix L, Haigle J, Morlière P, Mazière JC, Mazière C, Labrid C: Daflon as a cellular antioxidant and a membrane-stabilizing agent in human fibroblasts irradiated by ultraviolet A. *Photoderm Photoimmunol Photomed* 8:200–205, 1992
16. Djavaheri-Mergny M, Mazière JC, Santus R, Mora L, Mazière C, Auclair M, Dubertret L: Exposure to long wavelength ultraviolet radiation decreases processing of low density lipoproteins by cultured human fibroblasts. *Photochem Photobiol* 57:302–305, 1993
17. Yagi K: Lipid peroxides and human diseases. *Chem Phys Lipids* 45:337–351, 1987
18. Peterson GA: A simplification of the protein assay of Lowry *et al*, which is more generally applicable. *Anal Biochem* 83:346–356, 1977
19. Borenfreud E, Puerner JA: A simple quantitative procedure using monolayer cultures for cytotoxicity assays (HTD/NR-90). *J Tissue Cult Meth* 9:7–9, 1984
20. Bourre JM, Faivre A, Dumont O, Nouvelot A, Loudes C, Puymirat J, Tixier-Vidal A: Effect of polyunsaturated fatty acids on fetal mouse brain cells in culture in a chemically-defined medium. *J Neurochem* 41:1234–1242, 1983
21. Calder PC, Bond JA, Harvey DJ, Gordon S, Newsholme EA: Uptake and incorporation of saturated and unsaturated fatty acids into macrophage lipids and their effect upon macrophage adhesion and phagocytosis. *Biochem J* 269:807–814, 1990
22. Spector AA, York MA: Membrane lipid composition and cellular function. *J Lipid Res* 26:1015–1035, 1985
23. Ponec M, Weerheim A, Kempenaar J, Mommaas AM, Nugteren DH: Lipid composition of cultured human keratinocytes in relation to their differentiation. *J Lipid Res* 29:949–961, 1988
24. Biaglow JE, Varnes ME, Clark EP, Epp ER: The role of thiols in cellular response to radiation and drugs. *Radiation Res* 95:437–455, 1983
25. Kosugi K, Morel DW, DiCorletto PE, Chisolm GM: Toxicity of oxidized low density lipoprotein is selective for S phase of the cell cycle. *J Cell Physiol* 130:311–320, 1986
26. Gaboriau F, Morlière P, Marquis I, Moysan A, Gèze M, Dubertret L: Membrane damage induced in cultured human skin fibroblasts by UVA radiation. *Photochem Photobiol* 58:515–520, 1993
27. Esterbauer H: Lipid peroxidation products: formation, chemical properties and biological activities. In: Poli G, Cheeseman KH, Dianzani MU, Slater TF (eds.). *Free Radicals and Liver Injury*. IRL Press Limited, Oxford, England, 1985, pp 29–47
28. Mazière JC, Salmon S, Santus R, Candide C, Morlière P, Reyftmann JP, Mazière C, Dubertret L: Lipid oxidation and cellular functions: in vivo and in vitro models. In: Crastes de Paulet A, Douste-Blazy L, Paoletti R (eds.). *Free Radicals, Lipoproteins and Membrane Lipids*. NATO ASI Series, Plenum Press, New York, 1990, pp 327–342
29. Brasitus TA, Davidson NO, Schachter D: Variations in dietary triacylglycerol saturation alter the lipid composition and fluidity of rat intestinal plasma membrane. *Biochim Biophys Acta* 812:460–472, 1985
30. Christon R, Even V, Daveloose D, Léger C, Viret J: Modification of fluidity and lipid-protein relationship in pig intestinal brush-border membrane by dietary essential fatty acid deficiency. *Biochim Biophys Acta* 980:77–84, 1989
31. Chapkin RS, Akoh CC, Miller CC: Influence of dietary n-3 fatty acids on macrophage glycerophospholipid molecular species and peptidoleukotriene synthesis. *J Lipid Res* 32:1205–1213, 1991
32. Margolis LB: Cell interaction with model membranes. *Biochim Biophys Acta* 779:161–189, 1984
33. Callaghan T, Metezeau P, Gachelin H, Redziniak G, Milner Y, Goldberg ME: Modulation of the binding and endocytosis of concanavalin A by guinea pig keratinocytes: reversible antagonistic effects of cholesterol and phospholipid-liposomes. *J Invest Dermatol* 94:58–64, 1990
34. Parrish JA, Jaenicke KF, Anderson RR: Erythema and melanogenesis action spectra of normal human skin. *Photochem Photobiol* 36:187–191, 1982
35. Pearse AD, Gaskell SA, Marks D: Epidermal changes in human skin following irradiation with either UVB or UVA. *J Invest Dermatol* 88:83–87, 1987
36. Reed DJ: Regulation of reductive processes by glutathione. *Biochem Pharmacol* 37:7–13, 1986
37. Vessey DA, Lee KH, Blacker KL: Characterization of the oxidative stress in cultured human keratinocytes by treatment with peroxides. *J Invest Dermatol* 99:859–863, 1992
38. Tyrrell RM, Pidoux M: Correlation between endogenous glutathione content and sensitivity of cultured human skin cells to radiation at defined wavelengths in the solar ultraviolet range. *Photochem Photobiol* 47:405–412, 1988